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1	2663	transfect\$4) with retrovirus	USPAT; US-PGPUB; EPO; JPO; DERWENT	08:46	
2	453	neuroblastoma glioblastoma)	USPAT; US-PGPUB; EPO; JPO; DERWENT	08:47	
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US-PAT-NO:

5547932

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US 5547932 A

IDENTIFIER:

TITLE:

Composition for introducing nucleic acid complexes

into higher eucaryotic cells

DATE-ISSUED:

August 20, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP C	ODE COUNTRY
Curiel; David T.	Chapel Hill	NC	N/A	N/A
Birnstiel; Max L.	Vienna	N/A	N/A	AT
Cotten; Matthew	Vienna	N/A	N/A	AT
Wagner; Ernst	Langenzersdorf	N/A	N/A	AT
Zatloukal; Kurt	Vienna	N/A	N/A	AT
Plank; Christian	Vienna	N/A	N/A	AT
Oberhauser; Berndt	Vienna	N/A	N/A	AT
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Genentech, Inc.	San Francisco	CA	N/A	N/A	02

APPL-NO:

07/ 948357

DATE FILED: September 23, 1992

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS The present application is a continuation—in—part of U.S. application Ser. No. 07/937,788, filed Sep. 2, 1992, now abandoned which is a continuation—in—part of U.S. application Ser. No. 07/864,759, filed Apr. 7, 1992, which is a continuation—in—part of U.S. application Ser. No. 07/827,102, filed Jan. 30, 1992, now abandoned which is a continuation—in—part of U.S. application Ser. No. 07/767,788, filed Sep. 30, 1991 now abandoned. The present application is also a continuation—in—part of U.S. application Ser. No. 07/827,103, filed Jan. 30, 1992 now abandoned, and is a continuation—in—part of U.S. application Ser. No. 07/768,039, filed Sep. 30, 1991 now abandoned. The contents of each of these related applications is fully incorporated by reference

herein.

INT-CL: [06] C12Q001/70,C07H021/04 ,A01N063/00 ,C12N015/00 435/65, 435/69.1 , 435/91.4 , 435/91.41 , 435/240.2 , US-CL-435/252.3 , 435/267 , 435/6 , 536/23.5 , 536/24.5 , ISSUED: 424/93.1 , 424/93.2 , 424/93.6 , 424/520 , 935/32 , 935/57 , 935/71 US-CL-<u>435/456</u>, <u>424/520</u>, <u>424/93.1</u>, <u>424/93.2</u>, <u>424/93.6</u>, 435/252.3, **CURRENT**: 435/267, 435/458, 435/6, 435/69.1, 435/91.4, 435/91.41, <u>536/23.5</u>, <u>536/24.5</u> 435/6; 435/91.1; 435/7.2; 435/7.21; 435/7.23; 435/7.24; FIELD-435/69.1 ; 435/172.1 ; 435/172.3 ; 435/267 ; 435/5 ; OF-435/91.4; 435/91.41; 435/240.2; 435/252.3; 536/23.1; SEARCH: 536/23.4 ; 536/23.5-.51 ; 536/24.5 ; 930/220 ; 930/221 ; 935/22-24; 935/59; 935/60; 935/62; 935/76; 935/32; 935/57 ; 935/71 ; 935/63

REF-CITED:

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<u>5166320</u> November 1992	Wu et al.	530/395	N/A N/A
5225182 July 1993	Sharma	424/9	N/A N/A
5240846 August 1993	Collins et al.	435/240.1	N/A N/A

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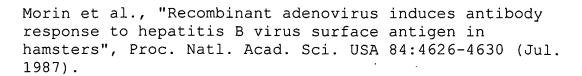
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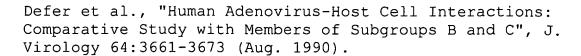
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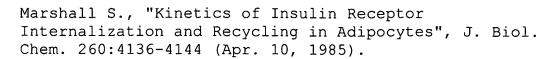
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Zhang & Nagaraja, "Differentiation of avian adenovirus type-II strains by restriction endonuclease fingerprinting", Am. J. Vet. Res. 50:1466-1470 (Sep. 1989).

ART-UNIT: 187

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ABSTRACT:

A composition for the transfection of higher eucaryotic cells, comprising complexes of nucleic acid, a substance having an affinity for nucleic acid and optionally an internalizing factor, contains an endosomolytic agent, e.g. a virus or virus component, which may be conjugated. The endosomolytic agent, which is optionally part of the nucleic acid complex, is internalized into the cells together with the complex and releases the contents of the endosomes into the cytoplasm, thereby increasing the gene transfer capacity. Pharmaceutical preparations, transfection kits and methods for introducing nucleic acid into higher eucaryotic cells by treating the cells with the composition are also disclosed.

75 Claims, 78 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 65

Drawing Description Text - DRTX:

B: Neuroblastoma cells.

Drawing Description Text - DRTX:

FIG. 31: Transfection of <u>neuroblastoma</u> cells with a 48 kb cosmid by means of biotin-streptavidin coupled adenovirus.

Detailed Description Text - DETX:

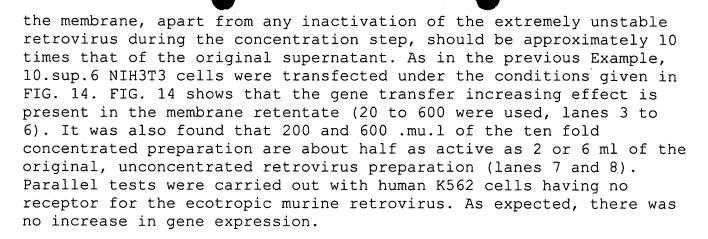
Other viruses, e.g. the coated viruses <u>Sendai</u>, HIV and some strains of Moloney leukaemia virus, or the uncoated viruses SV40 and polyoma, do not need a low pH for penetration into the cell; they can either bring about fusion with the membrane directly on the surface of the cell (<u>Sendai</u> virus, possibly HIV) or they are capable of triggering mechanisms for breaking up the cell membrane or passing through it. It is assumed that the viruses which are independent of pH are also capable of using the endocytosis route (McClure et al., 1990).

Detailed Description Text - DETX:

NIH3T3 cells were grown in DMEM medium with the addition of 10% FCS, 100 I.U./ml penicillin, 100 .mu.g/ml streptomycin and 2 mM glutamine. For the transfections, 5 to 7.times.10.sup.5 cells per T25 were plated out 18 to 24 hours before transfection. Immediately before transfection, the cells were placed in fresh medium and the various components used for transfection were added in the following order: Chloroquine (100 .mu.M, where stated), polylysine-transferrin-DNA complex and retrovirus preparation. The cells were then incubated for 4 hours at 37.degree. C., and the medium was changed and the cells were harvested 24 hours later. Extracts were prepared using three freeze/thaw cycles; aliquots of the extract, standardized for of protein content, were examined for luciferase activity as stated in the preceding Examples.

Detailed Description Text - DETX:

The virus preparation used in Example 9 was a crude, unfractionated supernatant of retrovirus expressing cells. In order to obtain evidence that the increase in the DNA <u>transfer</u> achieved with this virus preparation could actually be ascribed to the virus, the supernatant was subjected to the dialysis/concentration purification described above, the <u>retrovirus</u> supernatant (shown as RVS in the drawing) being concentrated by a factor 10. If the retrovirus is responsible for the increase, the activity retained by

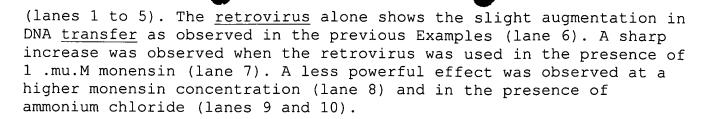


Detailed Description Text - DETX:

In order to rule out the possibility that the transfer of TfpL/pRSVL complexes into the cells can be ascribed to non-specific binding of polylysine to the retrovirus, and in order to clarify the entry mechanism further, the retrovirus was examined for its ability to transport plasmid DNA, complexed only with polylysine, into the cell. The quantity of polylysine used corresponds to the optimum amount determined earlier which brings about total condensation of the plasmid DNA and is similar to the quantity of the polylysine used with the polylysine-transferrin conjugate (Wagner et al., 1991a; the disclosure of which is fully incorporated by reference herein). The tests, the results of which are shown in FIG. 15, demonstrated that the reporter gene, in the absence of chloroquine, is not expressed either in the form of TfpL-pRSVL complexes or in the form of pL-pRSVL complexes (lanes 1 and 2). In the presence of the retrovirus, on the other hand, the reporter DNA applied as a TfpL complex was expressed, but not in the form of pL-DNA complex (see lanes 3 and 4 together with lanes 5 and 6). Moreover, the tests carried out showed that the presence of excess free transferrin resulted in the reduction in the DNA transfer facilitated by the retrovirus (lanes 7 and 8). These results support the proposition that interactions between transferrin and its receptor play an essential part in augmenting the DNA uptake effected by the retrovirus.

Detailed Description Text - DETX:

The experiments carded out in this Example were performed in order to examine the influence of the pH on the ability of retroviruses to augment gene transfer. The transfection experiments were carded out as in the preceding Examples. The two well-characterized inhibitors of endosome pH reduction, monensin and ammonium chloride, were used. The experimental results are shown in FIG. 16. The effect of the two substances on TfpL-DNA transfer was investigated and it was found that neither of the two substances can functionally replace chloroquine. However, a slight increase in the luciferase gene expression was found at higher ammonium chloride concentrations



Detailed Description Text - DETX:

c) Delivery of the Cosmid into Neuroblastoma Cells

Detailed Description Text - DETX:

Detailed Description Text - DETX:

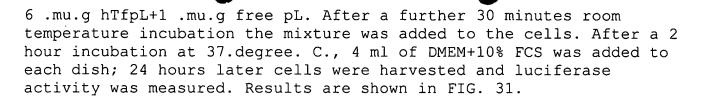
Example 21--Transfection of <u>Neuroblastoma</u> Cells with a 48 kb Cosmid in Presence of Adenovirus

Detailed Description Text - DETX:

b) Delivery of the Cosmid into Neuroblastoma Cells

Detailed Description Text - DETX:

Cells of a Neuroblastoma cell line designated GI-ME-N (Donti et al., 1988) (1.times.10.sup.6 cells per 6 cm dish) covered with 1 ml DMEM+2% FCS were incubated with TfpL/DNA complexes prepared as described in materials and methods section, containing the indicated quantities of hTfpL, free pL and DNA. As indicated, cell incubation mixtures included, in addition, either 100 .mu.M chloroquine (lanes 3 and 4) or 10 .mu.l adenovirus d1312 containing 5.times.10.sup.11 particles per ml, (lanes 5 and 6). The last two samples (indicated as StpL/Biotin) contained 1.5 .mu.l biotinylated adenovirus d1312 (1.times.10.sup.11 particles) incubated with streptavidin-polylysine (0.8 .mu.g prepared as in Example 19) for 30 minutes in 150 .mu.l HBS. 6 .mu.g DNA in 150 .mu.l HBS was then added to the sample for 30 minutes, room temperature, followed by 150 .mu.l HBS containing



Other Reference Publication - OREF:

Armentano et al., "Expression of human factor IX in rabbit hepatocytes by <u>retrovirus</u>-mediated gene <u>transfer</u>: Potential for gene therapy of hemophilia B", Proc. Natl. Acad. Sci. USA 87:6141-6145 (Aug. 1990).